THE MEASUREMENT OF *TRYPANOSOMA RHODESIENSE**

BY

J. W. W. STEPHENS, M.D. (CANTAB.), D.P.H.,

AND

H. B. FANTHAM, D.Sc. (LOND.), B.A. (CANTAB.)

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PLATE XIII

INTRODUCTION

The following paper contains the results of a biometric study of *Trypanosoma rhodesiense* (Stephens and Fantham).

This trypanosome, which is dimorphic, was described by us in July, 1910. It was considered to be a new species of trypanosome, producing Sleeping Sickness in man, since it could be distinguished morphologically by the fact that a certain percentage of short forms showed the nucleus either close to or even posterior to the blepharoplast, a feature which has never been recorded for *T. gambiense*, either before or since.

Otherwise, in *external* morphology *T. rhodesiense* closely resembles *T. gambiense*, for there are long, slender forms and short, stumpy forms, together with intermediate forms. These trypanosomes were figured by us (1910) in our original plate and are well shown in the accompanying coloured plate, for which we are greatly indebted to Lady Bruce.

METHODS

The blood-films used were quickly dried, fixed in absolute alcohol, and stained with a modified Romanowsky solution. Films of this nature contain trypanosomes most nearly approximating to the natural size. The flagellates suffer shrinkage in films fixed with sublimate-alcohol.

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One thousand specimens of the trypanosome have been measured after the manner introduced by Sir David Bruce for the differentiation of various trypanosomes. In this method the length of the median longitudinal axis, including the free flagellum, is determined as accurately as possible. We found it advisable to modify Bruce's method in some respects:—

(1) Instead of drawing the trypanosomes with a camera lucida, it is much easier to project them on a screen, using a photomicrographic apparatus in a dark room, and then to trace them in outline with a finely pointed pencil. The magnification is verified by projecting a millimetre scale in the same manner. The magnification adopted was 2,500 diameters, using a 2 mm. apochromatic objective and an 8 compensating ocular. This method not only saves much eyestrain in drawing, but is also much quicker.

(2) A more important modification consists in the actual mode of measuring the trypanosomes drawn on paper. Sir David Bruce uses for this purpose a pair of compasses, set at a fixed distance of 2 mm., his trypanosomes being magnified 2,000 times. There are, however, two objections to this method:—

(*a*) It cannot and does not give an accurate measurement, because the compass makes a series of 'jumps' and theoretically and actually the measurements given are always less than the true ones.

We can illustrate our objections perhaps by supposing that we have to measure the outline made by the teeth of an old saw. If the teeth are equal and the distance between the compass-points is equal to the depth of a tooth, then the course can be measured. If the depths of the teeth are unequal, then it will be impossible to get an accurate measurement by the compass method, though this can be accurately done by the 'tangent line' method. Although the curves of a trypanosome do not change their direction so acutely as the outline of a saw, yet the curves often do change their direction to some extent and the principle of the objection remains. We therefore used the method which we call the 'tangent line' method.

The requirements are:—(1) a piece of tracing paper on which a straight line is drawn in ink, (2) a pin, (3) a millimetre scale. The tracing paper is placed over the drawing of the trypanosome, which is seen through it. When the tracing paper is fixed by slight pressure of the pin placed on the ink line, the tracing paper can be rotated and the most tortuous curves followed with ease. One end of the ink line is placed on one end of the trypanosome. If the axis of the trypanosome curves, for example, at the nucleus, the pin is placed at this point and the paper is now rotated until the ink line coincides with the new direction of the axis. This is done as often as is necessary, and in fact the sharpest curves can be followed in this way, which is impossible by a compass, the points of which are at a fixed distance. Finally, the other end of the trypanosome is reached, the pin is placed there and the actual extent of the ink line traversed is measured by the millimetre scale. Further, the method has the advantage that it can equally well be applied to the measurement of any other curved line, for example, the axis of a spirochaete.

(*b*) Another objection to the compass method is that, if a start be made at the non-flagellar end of the trypanosome, it is uncertain that the finish will be exactly at the end of the flagellum. If not, there is always a portion of a compass distance which has to be guessed. With the tangent line method this is avoided, and the finish is exactly at the end.

The measurements could also be made by a self-registering rotameter ('map-measurer'), but we think that it is not quite such a convenient method for accurately following the curve.

It may be added that all the trypanosomes were outlined by one of us, and measured by the other.

MEASUREMENTS AND RESULTS

The following table gives the distribution, in respect to length, of 1,000 specimens of *T. rhodesiense* taken from various hosts, and measured in groups of 20 consecutive trypanosomes, neglecting only dividing forms.

TABLE I.—Distribution in respect to Length of 1000 Individuals of *Trypanosoma rhodesiense*

Animal.	In microns																												Average length
	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
Man	—	—	—	—	—	1	—	2	3	4	2	1	1	1	3	1	—	1	—	—	—	—	—	—	—	—	—	—	22·4
,, ...	—	1	—	1	3	—	2	2	3	—	1	2	1	—	2	1	1	—	—	—	—	—	—	—	—	—	—	—	20·4
,, ...	—	—	—	—	—	1	2	—	—	4	5	3	2	2	—	—	1	—	—	—	—	—	—	—	—	—	—	—	22·1
,, ...	1	—	1	1	—	3	3	2	2	2	—	1	—	1	1	1	—	—	1	—	—	—	—	—	—	—	—	—	19·8
,, ...	—	—	—	1	—	—	2	3	—	2	1	—	4	2	3	1	—	—	—	1	—	—	—	—	—	—	—	—	22·7
Monkey	—	—	—	—	—	1	—	2	3	1	3	—	2	2	3	—	2	—	1	—	—	—	—	—	—	—	—	—	23·2
,, ...	—	—	—	1	1	—	2	1	2	3	2	—	3	2	2	1	—	—	—	—	—	—	—	—	—	—	—	—	21·7
Horse...............	—	—	1	—	—	—	1	1	3	—	—	—	—	—	2	—	4	1	6	—	1	—	—	—	—	—	—	—	25·8
,, ...	—	—	1	—	1	1	3	1	1	—	2	—	1	3	1	1	4	—	—	—	—	—	—	—	—	—	—	—	22·4
Dog	—	—	2	—	2	—	4	2	4	3	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18·9
,, ...	—	—	—	—	2	—	—	3	9	1	—	1	1	—	1	—	2	—	—	—	—	—	—	—	—	—	—	—	20·9
Rabbit	—	—	—	—	—	—	2	1	4	1	2	4	4	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	22·0
,, ...	—	—	1	6	2	3	5	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16·8
Guinea-pig 25...	—	—	—	—	—	1	—	—	—	—	3	1	—	2	2	1	5	1	3	1	—	—	—	—	—	—	—	—	26·2
,, 25...	—	—	—	—	—	2	1	—	1	2	—	—	2	—	1	1	5	1	4	—	—	—	—	—	—	—	—	—	25·2
,, 21...	—	—	—	—	—	—	1	—	3	1	—	1	4	2	2	3	2	—	—	1	—	—	—	—	—	—	—	—	24·4
,, 24...	—	—	—	1	3	—	3	2	2	—	—	1	—	—	4	2	1	—	1	—	—	—	—	—	—	—	—	—	21·7
,, 24...	—	—	—	—	—	—	2	1	2	—	1	2	4	—	1	1	3	1	2	—	—	—	—	—	—	—	—	—	24·2
Mouse A	—	—	—	—	—	1	3	1	4	1	3	3	1	—	2	—	—	1	—	—	—	—	—	—	—	—	—	—	21·5
,, B	—	—	1	—	1	1	1	3	4	—	5	1	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20·4
Rat B 16	—	—	—	1	—	—	—	2	3	2	3	1	2	2	3	—	1	—	—	—	—	—	—	—	—	—	—	—	22·4
,, ...	—	—	—	—	—	1	1	1	2	3	5	2	1	2	—	2	—	—	—	—	—	—	—	—	—	—	—	—	22·0
,, ...	—	—	—	—	—	—	—	—	1	1	1	—	—	—	4	—	—	3	2	4	1	2	1	—	—	—	—	—	28·5
,, ...	—	1	2	2	3	3	2	3	3	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17·2

,, ...	—	—	—	2	2	1	3	1	5	3	—	—	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19·4
,, ...	—	—	—	—	—	—	—	2	—	—	1	4	1	2	1	1	3	3	1	1	—	—	—	—	—	—	—	—	25·5
,, ...	—	—	—	—	—	1	—	—	3	1	1	1	—	—	3	1	3	2	3	1	—	—	—	—	—	—	—	—	25·5
,, ...	—	—	—	—	1	—	1	—	—	2	2	3	2	1	5	—	1	1	1	—	—	—	—	—	—	—	—	—	23·9
,, ...	—	—	—	—	—	—	—	2	1	2	—	—	2	—	4	5	1	—	1	1	1	—	—	—	—	—	—	—	25·4
,, ...	—	—	—	—	—	—	2	—	4	—	2	4	1	2	2	1	1	1	—	—	—	—	—	—	—	—	—	—	23·1
,, ...	—	—	—	1	—	1	—	1	—	—	3	—	3	—	6	1	1	3	—	—	—	—	—	—	—	—	—	—	24·3
,, ...	—	1	1	1	2	3	1	3	2	2	1	—	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	19·0
Rat B 40	—	—	—	—	—	—	—	—	—	1	—	1	3	1	3	2	3	3	2	—	—	1	—	—	—	—	—	—	26·8
,, ...	—	—	—	—	—	—	—	—	—	—	—	2	—	2	—	8	2	2	2	2	—	—	—	—	—	—	—	—	27·4
Rat B 41	—	—	—	—	—	—	1	—	—	—	1	3	1	3	—	3	—	1	4	—	1	1	1	—	—	—	—	—	26·8
,, ...	—	—	—	—	—	—	—	—	—	—	1	—	3	2	2	—	2	1	4	4	—	1	—	—	—	—	—	—	27·9
Rat B 42	—	—	—	—	—	—	—	—	—	—	2	—	1	—	2	2	—	4	2	3	1	3	—	—	—	—	—	—	28·7
,, ...	—	—	—	—	—	—	—	—	—	—	1	2	—	3	—	2	2	1	1	4	1	1	—	1	1	—	—	—	28·6
,, ...	—	—	—	—	—	—	—	—	—	—	—	1	2	1	2	1	1	1	2	2	3	3	1	—	—	—	—	—	29·1
,, ...	—	—	—	—	—	—	1	1	4	1	1	1	—	1	2	2	2	2	1	1	—	—	—	—	—	—	—	—	24·4
Rat B 34	—	—	—	—	—	2	—	—	2	—	3	1	—	—	—	1	3	5	2	—	1	—	—	—	—	—	—	—	25·5
,, ...	—	—	—	—	—	—	3	—	—	1	1	—	3	—	—	—	3	4	1	—	1	1	1	—	—	—	—	1	26·8
,, ...	—	—	—	—	—	—	1	1	—	2	1	1	1	1	2	1	2	1	2	1	2	—	1	—	—	—	—	—	26·3
,, ...	—	—	—	—	2	1	3	—	2	—	—	2	—	2	—	2	1	2	2	1	—	—	—	—	—	—	—	—	23·5
Rat B 46	—	—	—	—	—	1	4	2	—	—	2	2	2	3	1	2	1	—	—	—	—	—	—	—	—	—	—	—	22·4
,, ...	—	—	—	—	1	1	1	4	2	—	2	—	—	3	3	3	—	—	—	—	—	—	—	—	—	—	—	—	22·2
,, ...	—	—	—	—	—	1	1	1	—	1	4	—	3	2	1	2	2	1	1	—	—	—	—	—	—	—	—	—	24·0
,, ...	—	—	—	—	1	2	2	1	2	1	—	1	—	3	—	2	4	1	—	—	—	—	—	—	—	—	—	—	22·9
,, ...	—	—	—	—	—	—	1	—	3	3	1	2	1	2	3	1	2	1	—	—	—	—	—	—	—	—	—	—	23·6
,, ...	—	—	—	1	2	2	2	1	1	—	1	1	1	1	5	—	1	1	—	—	—	—	—	—	—	—	—	—	21·8
Total	1	3	10	19	29	35	67	54	92	51	74	56	68	59	85	61	72	50	52	28	13	13	5	1	1	—	—	1	
Percentages...	0·1	0·3	1·0	1·9	2·9	3·5	6·7	5·4	9·2	5·1	7·4	5·6	6·8	5·9	8·5	6·1	7·2	5·0	5·2	2·8	1·3	1·3	0·5	0·1	0·1	—	—	0·1	

In the following table the foregoing data are summarised to show the average, maximum and minimum lengths in the different hosts on various days of infection.

TABLE II.—Measurements of the Length of *Trypanosoma rhodesiense*

Animal	Day of infection	In microns		
		Average length	Maximum length	Minimum length
Man	80 approx.	22·4	29·0	17·0
,, ...	117 ,,	20·4	28·0	13·0
,, ...	117 ,,	22·1	28·0	17·0
,, ...	123 ,,	19·8	30·0	12·0
,, ...	123 ,,	22·7	31·0	15·0
Monkey	9	23·2	30·0	17·0
,, ...	10	21·7	27·0	15·0
Horse...............	31	25·8	32·0	14·0
,, ...	32	22·4	28·0	14·0
Dog	4	18·9	22·0	14·0
,, ...	7	20·9	28·0	16·0
Rabbit	25	22·0	27·0	18·0
,, ...	38	16·8	20·0	14·0
Guinea-pig 25...	18	26·2	31·0	17·0
,, 25...	18	25·2	30·0	17·0
,, 21...	43	24·4	31·0	18·0
,, 24...	56	21·7	30·0	15·0
,, 24...	58	24·2	30·0	18·0
Mouse A	6	21·5	29·0	17·0
,, B	7	20·4	25·0	14·0
Rat B 16	4	22·4	28·0	15·0
,, ...	5	22·0	27·0	17·0
,, ...	6	28·5	34·0	20·0
,, ...	7	17·2	22·0	13·0
,, ...	8	19·4	25·0	15·0
,, ...	9	25·5	31·0	19·0
,, ...	10	25·5	31·0	17·0
,, ...	11	23·9	30·0	16·0
,, ...	12	25·4	32·0	19·0
,, ...	12	23·1	29·0	18·0
,, ...	13	24·3	29·0	15·0
,, ...	13	19·0	27·0	13·0
Rat B 40	3	26·8	33·0	21·0
,, ...	3	27·4	31·0	23·0
Rat B 41	3	26·8	34·0	18·0
,, ...	3	27·9	33·0	22·0
Rat B 42	7	28·7	33·0	22·0
,, ...	7	28·6	36·0	22·0
,, ...	7	29·1	34·0	23·0
,, ...	7	24·4	31·0	18·0
Rat B 34	11	25·5	32·0	17·0
,, ...	11	26·8	39·0	18·0
,, ...	11	26·3	34·0	18·0
,, ...	11	23·5	31·0	16·0
Rat B 46	12	22·4	28·0	17·0
,, ...	12	22·2	27·0	16·0
,, ...	12	24·0	30·0	17·0
,, ...	12	22·9	29·0	16·0
,, ...	12	23·6	29·0	18·0
,, ...	12	21·8	29·0	15·0
		23·6	39·0	12·0

On comparing these results with those obtained by Sir David Bruce for 1,000 *T. gambiense* and 1,000 *T. brucei* respectively, we get the following results:—

	In microns		
	Average length	Maximum length	Minimum length
T. rhodesiense	23·6	39	12
T. gambiense	22·1	33	13
T. brucei	23·2	38	13

From this table it is seen that the measurements of *T. rhodesiense* are practically the same as those of *T. brucei*, but differ from those of *T. gambiense*.

The average length of *T. rhodesiense* in man and other species of animals, summarised from Table I, is as follows:—

TABLE III

Animal	In microns		
	Average length	Maximum length	Minimum length
Man...........................	21·5	31·0	12·0
Monkey	22·4	30·0	15·0
Horse	24·1	32·0	14·0
Dog	19·9	28·0	14·0
Rabbit	19·4	27·0	14·0
Guinea-pig	24·3	31·0	15·0
Mouse	21·0	29·0	14·0
Rat	24·5	39·0	13·0

On comparing figures obtained from Table III with those from similar hosts in the case of *T. gambiense*, measured by Bruce, we get the following results:—

TABLE IV

	Average length	Maximum length	Minimum length
	μ	μ	μ
Man—			
T. gambiense	24·3	33·0	15·0
T. rhodesiense	21·5	31·0	12·0
Monkey—			
T. gambiense	22·4	31·0	15·0
T. rhodesiense	22·4	30·0	15·0
Rat—			
T. gambiense	22·4	32·0	13·0
T. rhodesiense	24·5	39·0	13·0

This table also appears to indicate that there are some differences in size between *T. gambiense* and *T. rhodesiense.*

If now the 1,000 *T. rhodesiense* are divided according to length into three groups—(*a*) short and stumpy forms of 13 to 21 microns, (*b*) intermediate forms of 22 to 24 microns, and (*c*) long and slender forms of 25 microns and upwards (as has been done by Sir David Bruce in his researches on trypanosomes), and comparison of them with Bruce's results for *T. gambiense* and *T. brucei* be made, the following percentage distributions are obtained:—

TABLE V

	Short and stumpy, 13—21 μ	Intermediate, 22—24 μ	Long and slender, 25—39 μ
	per cent.	per cent.	per cent.
T. gambiense	51·2	23·1	25·7
T. brucei	32·8	25·5	41·7
T. rhodesiense	36·1	19·8	44·1

We note that *T. rhodesiense* is richest in long and slender forms and poorest in intermediate forms.

If the percentages in the three groups are calculated for (i) each of the hosts infected with *T. gambiense* recorded in Bruce's Table III, and for (ii) each of the hosts infected with *T. rhodesiense* recorded in our Table I, then large variations are found to occur. Thus, from a comparison of 1,000 *T. gambiense,* measured from seven species of animals by Bruce, on a variety of days, and 1,000 *T. rhodesiense,* measured by us from eight species of animals on a variety of days, the following results are obtained:—

TABLE VI

	T. gambiense	*T. rhodesiense*
μ	per cent.	per cent.
13—21	32·0 to 82·1	28·0 to 80
22—24	14·3 to 33·3	7·5 to 37·5
25—39	3·6 to 52·0	5·0 to 57·5

Also the following table summarises the variation in 240 *T. rhodesiense* from the same rat (Table I, Rat B 16) from the 4th to the 13th day of infection.

TABLE VII

	T. rhodesiense (Rat B 16).
μ	per cent.
13—21	10 to 95
22—24	5 to 40
25—39	0 to 85

Thus it is clear that extreme variations in the length of the trypanosome are found in the different hosts, and on different days of infection in the same host, on examining the trypanosome in samples of 20.

If, again, a study of the distribution of 1,000 *T. gambiense*, 1,000 *T. rhodesiense*, and 1,000 *T. brucei** is made by the more usual method of quartiles or octiles, the following results are obtained:—

TABLE VIII

	125th	250th	375th	500th	625th	750th	875th
	μ	μ	μ	μ	μ	μ	μ
T. gambiense	18	19	20	21	23	25	27
T. rhodesiense	18	20	22	24	26	27	29
T. brucei	18	20	22	24	25	27	29

From this table it is seen that the measurements of *T. rhodesiense* and *T. brucei* are almost the same, but that they again differ from those of *T. gambiense*.

Our results are represented graphically in Chart 1:—

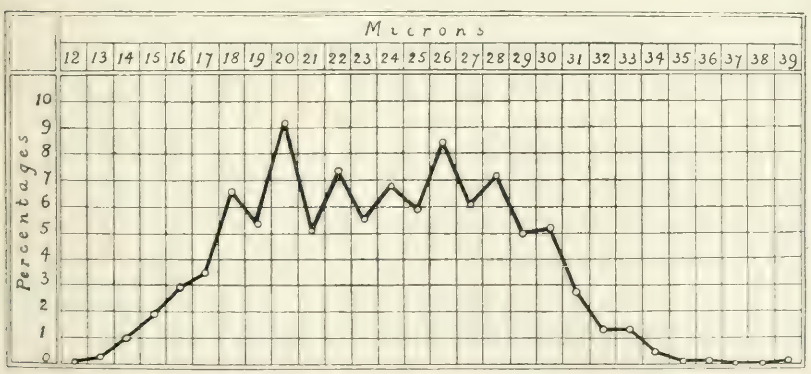

CHART 1.—Curve representing distribution, by percentages in respect to length, of 1000 specimens of *T. rhodesiense*, from various hosts.

* The figures for *T. brucei* have been deduced as accurately as possible from Bruce's curve (1911).

We also give a chart of 600 *T. rhodesiense* taken from the same species of host, namely, rats:—

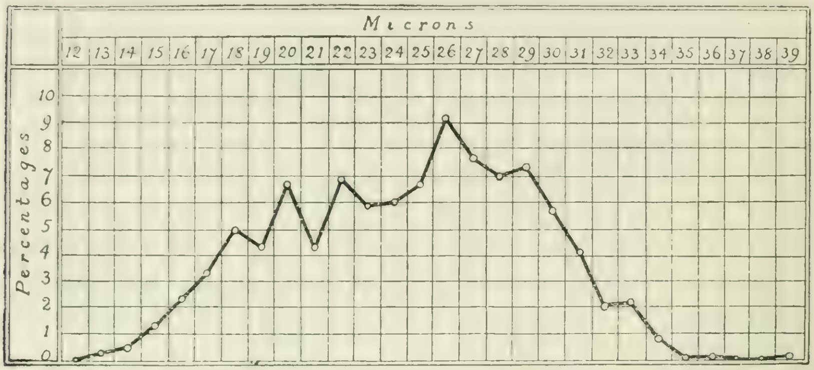

CHART 2.—Curve representing distribution, by percentages in respect to length, of 600 specimens of *T. rhodensiense* from rats. (See Addendum.)

If we now consider the graphic representation of our measurements of *T. rhodesiense*, as seen in Charts 1 and 2, and compare them with Bruce's curves of *T. gambiense* and *T. brucei*, we note the following points:—

While *T. gambiense* presents a curve with a single marked peak at 20μ, *T. rhodesiense* presents a series of small irregular peaks extending from 18μ to 28μ, with the highest peaks at 20μ and 26μ. In the case of *T. brucei* there is a slightly irregular curve extending from 18μ to 26μ, with a well-marked peak at 24μ.

Considering the three curves together, we note again that *T. rhodesiense* appears to be different from *T. gambiense*, but that the difference from *T. brucei* is slight.

DISCUSSION OF RESULTS

(1) We consider that a sample of 20 trypanosomes, at least in the case of dimorphic species like *T. rhodesiense*, from a particular slide on a particular day is too small, as the average length obtained in this way may vary in extreme cases between 24·4μ and 29·1μ (see Table I, Rat B 42).

(2) The day of infection on which the measurement is taken is very important, for, as we have seen in Table VII, on one day 10 % of stumpy forms may be found, on another day 95 %. This must, we think, be due to an actual change in the number of

trypanosomes of any particular length present, and not to an error of measurement.

(3) It is probable also that the host from which the trypanosome is taken is an important factor. It is difficult to be quite certain of this, because the variation may be due to the cause just stated, namely, the day of infection.

(4) However, giving these sources of error due weight, we think that the fact that there is a general resemblance between the curves representing the measurements of these three trypanosomes (*T. gambiense, T. rhodesiense, T. brucei*) shows that the method is a trustworthy one.

(5) The measurements of *T. rhodesiense* are much closer to those of *T. brucei* than to those of *T. gambiense*. We do not consider, however, that identity of measurement would necessarily imply identity of species. We still believe that the difference in internal morphology, namely, the presence of the posterior nucleus, is sufficient to separate *T. rhodesiense* both from *T. gambiense* and *T. brucei*.

(6) We think, however, that in the future, in order to get as accurate results as possible, it will be necessary on any particular day to measure larger samples than 20 trypanosomes. How large these samples must be, it is, at present, impossible to say, for we have not the requisite data. This is a point we propose shortly to investigate. At present we would suggest that, in order to eliminate unknown possible variations due to the use of different hosts, samples should always be taken from the *same* animal, and as we have shown that there are large variations on different days, samples should be taken on *every* day of the infection. Tame rats would appear to be the most suitable animals, as they are susceptible to the large majority of pathogenic trypanosomes. (See Addendum.)

Mr. Walter Stott, Honorary Statistician to the Liverpool School of Tropical Medicine, has kindly examined our figures and curves, and is of opinion that, on the whole, the data at present available are insufficient to enable statistical criticism to be applied, as there are no standard curves for comparison.

We propose, therefore, shortly to investigate the subject further from the various additional points of view that we have indicated.

Addendum, April 29, 1912.—Since writing the preceding we have completed a fresh series of measurements of *Trypanosoma rhodesiense* from a single rat, beginning with the first day of infection, and measuring 100 trypanosomes per day during 10 consecutive days of infection. We have thus obtained measurements of 1,000 trypanosomes from the same rat. On representing the results graphically, it was found that the curve resembled that of Chart 2 (for 600 trypanosomes from rats), rising with slight irregularities to a peak at 26μ (as does the curve of Chart 2), then falling rapidly and ending at 34μ.

Our remarks on pp. 140, 141 appear to be justified, but detailed discussion must be deferred.

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EXPLANATION OF PLATE XIII

Various forms of *Trypanosoma rhodesiense*, drawn at a magnification of 2,000 diameters.

Note that some of the short and stumpy forms have the nucleus posterior.

PLATE XIII

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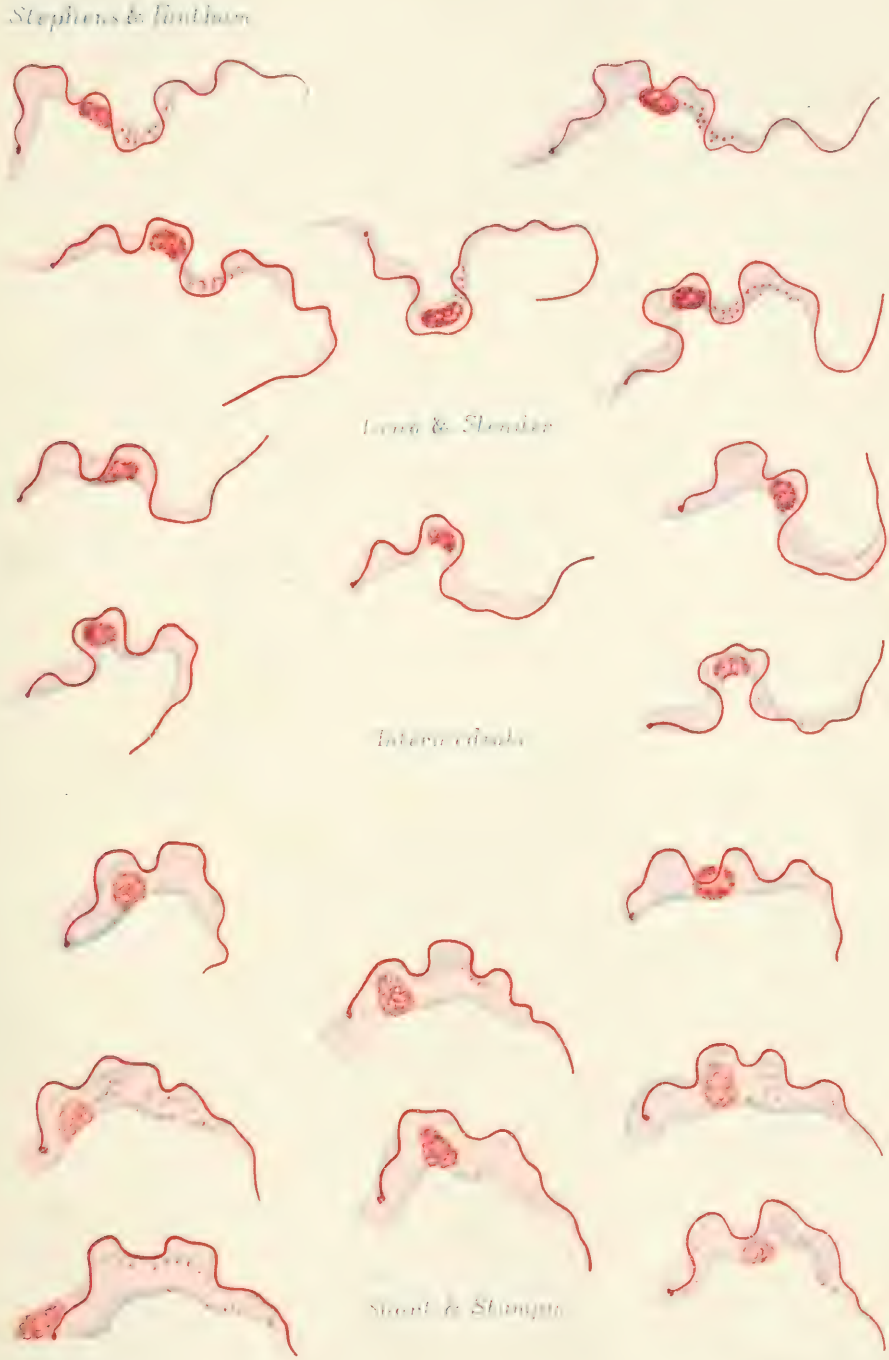

M.E. Bruce, del
M.P. Parker, lith.

E. Wilson, Cambridge

TRYPANOSOMA RHODESIENSE.